Journal of Food and Drug Analysis, Vol. 17, No. 2, 2009, Pages 116-122 藥物食品分析 第十七卷 第二期

Effect of Harvest Time on Saponins in Yam (*Dioscorea pseudojaponica* Yamamoto)

JAU-TIEN LIN[1], SU-LIN CHEN[2], SHIH-CHUAN LIU[2] AND DENG-JYE YANG[2]*

[1.] *Department of Applied Chemistry, Chung Shan Medical University, Taichung , Taiwan (R.O.C.)*

[2.] *School of Health Diet and Industry Management, Chung Shan Medical University, Taichung, Taiwan (R.O.C.)*

(Received: May 29, 2008; Accepted: December 15, 2008)

ABSTRACT

Steroidal saponins including furostanol and spirostanol glycosides are the important bioactive compounds in yams. In the study, the content of individual saponin in varied organs of yam (*Dioscorea pseudojaponica* Yamamoto) harvested from November to March of the next year (the harvest season) were determined. Results showed that total saponin levels in yam organs (except rhizophor) harvested at various time were in the order: January > December > February > November > March. Saponin contents in rhizophor gathered in December were higher than those obtained in January. The highest total amounts of saponins among various organs were in the order: tuber cortex (619.79 μg/g dw) > tuber flesh (247.84 μg/g dw) > rhizophor (32.19 μg/g dw) > leaf (26.57 μg/g dw) > vine (25.06 μg/g dw).

Key words: furostanol glycoside, spirostanol glycoside, steroidal saponin, yam (*Dioscorea* spp), harvest time

INTRODUCTION

Steroidal saponins including furostanol and spirostanol glycosides are important functional compounds in yam (*Dioscorea* spp.)[1-3]. Many researches demonstrated that steroidal saponins had anti-carcinogenic[4], anti-thrombotic[5], anti-viral[6], hemolytic[7], hypocholesterolemic[8] and hypoglycemic effects[9]. The aglycone (sapogenin) of yam steroidal saponins has also been used to produce steroidal drugs[10, 11].

Dinan *et al.*[12] and Lin and Yang[13] described that notable variation of saponin quantity could be found in plant organs; moreover, the cultivar, the age, or the geographic locality of the plant could also influence the saponin contents greatly[12]. The harvest time should also affect the saponin contents in plants. There were, however, no thorough report about the effect of harvest time on saponins in yams. In our previous study[3], six steroidal saponins consisting of three furostanol glycosides and three spirostanol glycosides were isolated from the tuber flesh of yam (*Dioscorea pseudojaponica* Yamamoto) and it was also found the tuber cortex contains abundant saponins[13]. The yam is Taiwanese native cultivar whose harvest time, in general, is from November to March of the next year[14]. In this investigation, we surveyed the saponin contents in yam organs (leaf, rhizophor, tuber flesh, tuber cortex and vine) that were harvested during the five months from the same farm. The influence of air temperature on yam saponins was also discussed.

* Author for correspondence. Tel: +886-4-24730022 ext. 11867; Fax: +886-4-23248188; Email: djyang@csmu.edu.tw

MATERIAL AND METHODS

I. *Yam Samples*

The leaves, vines, rhizophores (ca 1 cm in diameter and ca 4 cm in length) and tubers (white cortex and flesh, ca 4 cm in diameter and ca 130 cm in length) of yam plant (*D. pseudojaponica* Yamamoto) were harvested from the same farm randomly in Cidu District, Keelung City, Taiwan between Nov. 2006 and Mar. 2007. The yams were cultivated at the end of Mar. 2006. Their growing conditions were identical except for the harvest time. Each sample was collected about 10 kg in each month within the 5 months. The tubers were peeled and the cortices were gathered. The flesh of yam tubers and rhizophores were cut into 4 mm thick slices with a slicer. The leaves were took off from yam vines and pooled. The vines were cut into 2 cm of length. All of these samples were lyophilized in a freeze-dryer (Vastech Scientific Co., Ltd., Taipei, Taiwan), ground to flour and passed through 40 mesh standard sieve before experiment.

II. *Chemicals*

26-*O*-*β*-D-glucopyranosyl-22*α*-methoxyl-(25*R*)-

Journal of Food and Drug Analysis, Vol. 17, No. 2, 2009

furost-5-en-3*β*, 26-diol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-{[*α*-L-rhamnopyranosyl-(1→4)]-*O*-[*α*-L-rhamnopyranosyl-(1→4)]}-*β*-D-glucopyranoside (1), methyl protodioscin (2), methyl protogracillin (3), (25*R*)-spirost-5-en-3*β*-ol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-{[*α*-L-rhamnopyranosy-(1→4)]-*O*-[*α*-L-rhamnopyranosyl-(1→4)]}-*β*-D-glucopyranoside (4), dioscin (5) and gracillin (6) were isolated by the method as detailed in our previous report[1]. Their structures were showed in Figure 1. Methanol and *n*-butanol were purchased from Tedia Co. (Fairfield, OH, USA). Zorbax SPE C18 cartridges (200 mg) were obtained from Agilent Technologies, Inc. (Palo Alto, CA, USA). Deionized water was prepared by Ultrapure™ water purification system (Lotun Co., LTD. Taipei, Taiwan); it was degassed under vacuum and filtered through a 0.2 μm membrane filter (Millipore Co., Bedford, MA, USA).

III. *Extraction of Yam Saponins*

The method of yam saponins extraction was described by Lin and Yang[13]. The C18 cartridges (200 mg) were conditioned with 3 mL methanol and 3 mL of 50% methanol/water in advance. Fifty grams of each yam sample were extracted with 1 L methanol for 24 h at 25°C. The extract was filtered and the solvent was removed using a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan) at 30°C. The residue was then resuspended in 25 mL distilled water and partitioned against 25 mL *n*-butanol 3 times to yield saponin extract. After washing 3 times with 50 mL distilled water, the extract solvent was removed in a rotary evaporator at 45°C. The dried extract was then dissolved in 1 mL methanol, diluted with equal amount of water and loaded onto a C18 cartridge.

The cartridge was washed using 3 mL water and 3 mL of 50% methanol/water in turn; the saponins were then eluted with 3 mL of 80% methanol/H_2O. The eluate was concentrated to dryness in a rotary evaporator at 35°C and re-dissolved in 1 mL methanol prior to HPLC analysis.

IV. *HPLC Analysis of Yam Saponins*

A *PrimeLine*™ Gradient Model 500G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA, USA) outfitted with an Alltech ELSD 3300 evaporative light scattering detector (ELSD) (tube temperature, 75°C; air flow rate, 2.8 L/min) (Alltech Associates Inc., Deerfield, Ireland) was used for the analysis of yam saponins. The analytical condition was adopted from Yang *et al.*[15] A Luna C18 column (4.6 mm i.d. × 250 mm, 5 μm

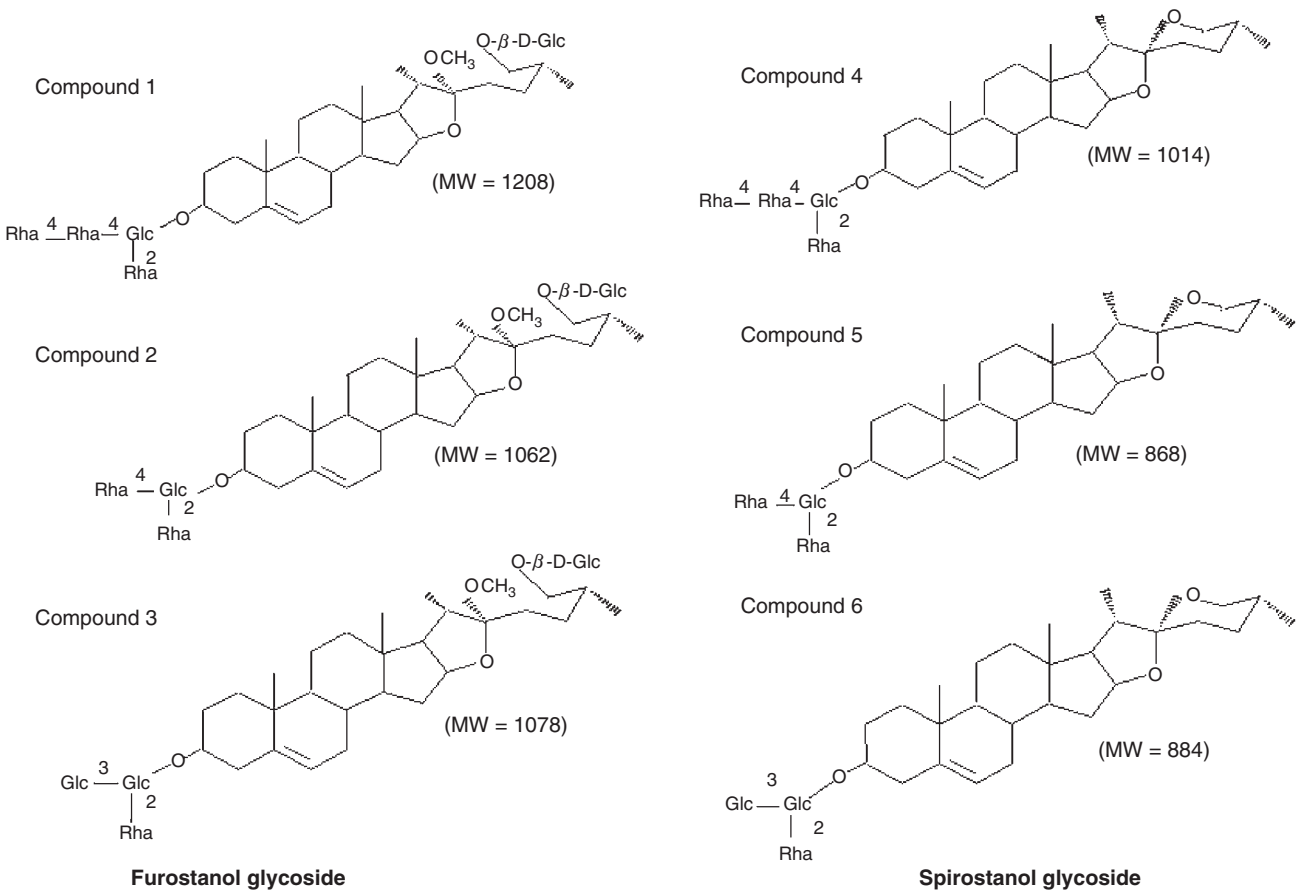

Figure 1. Chemical structures of yam saponins.

particle size) (Phenomenex, Torrance, CA, USA) was used for saponins separation; a step gradient solvent system consisting of methanol and deionized H_2O, 62/38 (v/v) in the first 20 min and 71/29 (v/v) from 21 to 65 min at a flow rate of 1 mL/min, was used for saponins elution. Data processing was conducted using a Chem-Win computer software system (Shuen-Hua Co., Taipei, Taiwan).

V. *Statistical Analysis*

The quantitative analyses of the yam saponins were performed in triplicate and the mean values were calculated. Statistical analyses of the data were done by the analysis of variance and Duncan's test procedures were dispensed to evaluate the significance between means, at a level of $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows that the tuber fleshes of yam gathered in Jan. 2007 contained the highest content of saponin; the second to fourth was those harvested in Dec. 2006, Feb. 2007 and Nov. 2006. The lowest amount of saponin was found in that harvested in Mar. 2007. Though the saponin contents in tuber cortices and fleshes had a similar trend, the contents of total furostanol glycosides in tuber cortices collected in Jan. 2007 and Dec. 2006 did not showed significant difference (Table 2). Yam tuber cortices had higher saponin contents than their homologous fleshes by 2.50- (collected in Jan. 2007) to 2.89- fold (collected in Mar. 2007) (Tables 1 and 2). Figure 2 shows the chromatograms of saponin extracts of yam tuber cortices collected at varied time. Milgate and Roberts[16] indicated that saponin could inhibit the growth of mold and help protect the plant from insect attack. Yam tuber is a reproductive organ; its cortex should play a principal role to resist the injurious factors in growing conditions, e.g., the insect pests, climatic change and so on. Therefore, tuber cortex should produce more secondary metabolites including saponin. Pęksa *et al*.[17] also found that unpeeled potato tuber had much higher glycoalkaloid (α-solanine and α-chaconine), the steroidal alkaloid with a similar structure to yam saponin, than the peeled one, which indicated that the cortex possess rich bioactive compounds.

Yam rhizophor, which is highly charactristic tuber-like organ on the presence of decussately arranged sporophylls. Table 3 indicates that all of the rhizophor samples lacked compounds 1 and 4, and even compounds 3 and 6 could not be measured in those harvested in Nov. 2006 and Mar. 2007, whose saponin quantities were lower than others. The saponin content in rhizophor collected in Dec. 2006 and Jan. 2007 did not show any significant difference, though the one collected in Dec. 2006 had the highest saponin content.

Tables 4 and 5 show that compounds 1 and 4 did not exist in all leaf and vine samples; moreover, those harvested in Nov. 2006, Feb. 2007 and Mar. 2007 contained compounds 2 and 5 merely. Both of the leaf and vine samples obtained in Jan. 2007 contained the highest saponin amounts; nevertheless, their amounts were lower than those in other yam organs, the tuber cortex and flesh especially. Kraverts *et al*.[18] indicated that saponins could not be found in leaf of onion but they exist in the fruit and seed, the reproductive organs.

Many researches[19-22] indicated that harvest time would affect the chemical composition and yield of crops.

Table 1. Saponin contents in yam tuber fleshes

Compounds	Saponin contents (μg/g dw)[a]				
	Harvest time				
	Nov. 2006	Dec. 2006	Jan. 2007	Feb. 2007	Mar. 2007
1	43.15 ± 2.35 D[b]	50.54 ± 2.91 B	53.90 ± 3.21 A	45.34 ± 3.41 C	38.76 ± 2.47 E
2	40.28 ± 2.18 D	55.63 ± 3.12 A	54.65 ± 2.37 B	42.72 ± 3.02 C	36.42 ± 2.66 E
3	22.35 ± 2.41 E	29.94 ± 2.51 B	31.54 ± 2.17 A	25.28 ± 1.85 C	24.38 ± 2.29 D
Total furostanol glycosides	105.78 ± 6.94 D	136.11 ± 8.54 B	140.09 ± 7.75 A	113.34 ± 8.28 C	99.56 ± 7.42 E
4	32.93 ± 1.87 C	37.07 ± 2.33 A	37.57 ± 2.54 A	34.27 ± 1.85 B	28.62 ± 2.36 D
5	39.17 ± 2.04 B	47.34 ± 2.18 A	46.94 ± 1.83 A	38.74 ± 2.63 B	33.44 ± 2.15 C
6	15.57 ± 2.05 C	21.38 ± 2.31 B	23.24 ± 3.04 A	16.62 ± 2.18 C	13.52 ± 1.27 D
Total spirostanol glycosides	87.67 ± 5.96 D	105.79 ± 6.82 B	107.75 ± 7.41 A	89.63 ± 6.66 C	75.58 ± 5.78 E
Total saponins	193.45 ± 12.90 D	241.9 ± 15.36 B	247.84 ± 15.16 A	202.97 ± 14.94 C	175.14 ± 13.20 E

[a]. All values are mean ± SD obtained by triplicate analyses.
[b]. Values bearing different letters in the same row are significantly different ($p < 0.05$).

Journal of Food and Drug Analysis, Vol. 17, No. 2, 2009

Table 2. Saponin contents in yam tuber cortices

Compounds	Saponin contents (μg/g dw)[a]				
	Harvest time				
	Nov. 2006	Dec. 2006	Jan. 2007	Feb. 2007	Mar. 2007
1	139.88 ± 9.23 C[b]	162.45 ± 9.91 A	162.36 ± 11.17 A	147.36 ± 7.96 B	132.65 ± 10.37 D
2	148.64 ± 10.04 C	159.38 ± 9.64 A	160.42 ± 10.52 A	154.21 ± 8.79 B	141.84 ± 9.64 C
3	79.41 ± 4.89 D	89.57 ± 5.08 A	87.34 ± 6.23 B	83.41 ± 6.03 C	73.81 ± 4.42 E
Total furostanol glycosides	367.93 ± 24.16 C	411.40 ± 24.63 A	410.12 ± 27.92 A	384.98 ± 22.78 B	348.30 ± 24.43 D
4	62.38 ± 3.79 D	73.25 ± 3.98 B	75.66 ± 4.47 A	70.32 ± 4.48 C	56.60 ± 3.52 E
5	74.53 ± 5.03 D	83.26 ± 5.45 B	87.71 ± 3.81 A	81.95 ± 4.95 C	67.72 ± 4.24 E
6	39.92 ± 3.36 C	47.52 ± 2.84 A	46.30 ± 3.63 B	40.39 ± 3.55 C	34.08 ± 2.99 D
Total spirostanol glycosides	176.83 ± 12.18 D	204.03 ± 12.27 B	209.67 ± 11.91 A	192.66 ± 12.98 C	158.40 ± 10.75 E
Total saponins	544.76 ± 36.34 D	615.43 ± 36.90 B	619.79 ± 39.83 A	577.64 ± 35.76 C	506.70 ± 35.18 E

[a]. All values are mean ± SD obtained by triplicate analyses.
[b]. Values bearing different letters in the same row are significantly different ($p < 0.05$).

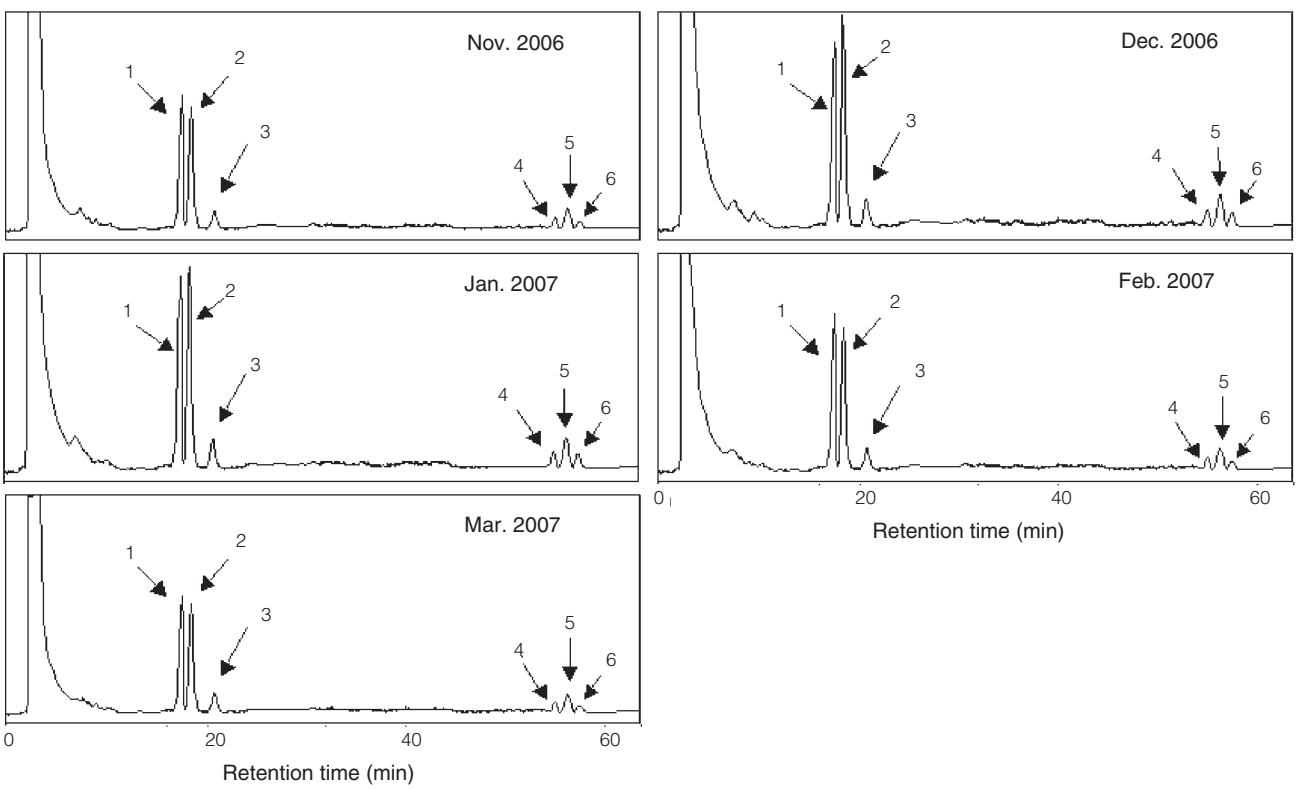

Figure 2. HPLC chromatograms of saponin extracts of yam tuber cortices harvested from various months. HPLC conditions: column, Luna C-18 (4.6 mm i.d. × 250 mm, 5 μm); mobile phase, MeOH/ H_2O = 62/38 (v/v) from 0 to 20 min and 71/29 (v/v) from 21 to 65 min; flow rate, 1 mL/min; detection, evaporative light scattering detector (ELSD) (tube temperature, 75°C; gas flow rate, 2.8 L/ min).
Compounds: (1) 26-*O*-*β*-D-glucopyranosyl-22*α*-methoxyl-(25*R*)-furost-5-en-3*β*, 26-diol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-{[*α*-L-rhamnopyranosyl-(1→4)]-*O*-[*α*-L-rhamnopyranosyl-(1→4)]}-*β*-D-glucopyranoside, (2) methyl protodioscin, (3) methyl protogracillin, (4) (25*R*)-spirost-5-en-3*β*-ol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-{[*α*-L-rhamnopyranosy-(1→4)]-*O*-[*α*-L-rhamnopyranosyl-(1→4)]}-*β*-D-glucopyranoside, (5) dioscin, (6) gracillin.

Table 3. Saponin contents in yam rhizophors

Compounds	Saponin contents (μg/g dw)[a]				
	Harvest time				
	Nov. 2006	Dec. 2006	Jan. 2007	Feb. 2007	Mar. 2007
1	ND[b]	ND	ND	ND	ND
2	6.05 ± 1.27 C[c]	8.23 ± 1.83 A	8.09 ± 2.03 AB	7.23 ± 1.71 B	5.22 ± 0.84 C
3	ND	6.91 ± 1.02 A	7.14 ± 0.81 A	2.12 ± 0.47 B	ND
Total furostanol glycosides	6.05 ± 1.27 C	15.14 ± 2.85 A	15.23 ± 2.84 A	9.35 ± 2.18 B	5.22 ± 0.84 C
4	ND	ND	ND	ND	ND
5	7.54 ± 2.08 C	10.88 ± 1.81 A	10.36 ± 2.05 A	8.64 ± 1.15 B	6.05 ± 1.22 D
6	ND	6.17 ± 0.88 A	5.93 ± 0.76 A	ND	ND
Total spirostanol glycosides	7.54 ± 2.08 B	17.05 ± 2.69 A	16.29 ± 2.81 A	8.64 ± 1.15 B	6.05 ± 1.22 C
Total saponins	13.59 ± 3.35 C	32.19 ± 5.54 A	31.52 ± 5.65 A	17.99 ± 2.86 B	11.27 ± 2.06 C

[a] All values are mean ± SD obtained by triplicate analyses.
[b] ND = not detected.
[c] Values bearing different letters in the same row are significantly different ($p < 0.05$).

Table 4. Saponin contents in yam leaves

Compounds	Saponin contents (μg/g dw)[a]				
	Harvest time				
	Nov. 2006	Dec. 2006	Jan. 2007	Feb. 2007	Mar. 2007
1	ND[b]	ND	ND	ND	ND
2	5.17 ± 0.75C[c]	6.89 ± 0.67A	6.82 ± 0.84 A	5.54 ± 0.92 B	4.25 ± 0.71D
3	ND	6.74 ± 0.69 A	6.84 ± 1.02 A	ND	ND
Total furostanol glycosides	5.17 ± 0.75B	13.63 ± 1.36 A	13.66 ± 2.27 A	5.54 ± 0.92 B	4.25 ± 0.71 C
4	ND	ND	ND	ND	ND
5	4.18 ± 0.56 B	5.96 ± 0.89 A	6.37 ± 1.03 A	6.04 ± 1.02 A	3.29 ± 0.45 C
6	ND	6.13 ± 1.17 B	6.54 ± 1.25 A	ND	ND
Total spirostanol glycosides	4.18 ± 0.56 C	12.09 ± 2.16 A	12.91 ± 2.28 A	6.04 ± 1.02 B	3.29 ± 0.45 C
Total saponins	9.35 ± 1.31 C	25.72 ± 3.42 A	26.57 ± 4.55 A	11.58 ± 1.94 B	7.54 ± 1.16 C

[a] All values are mean ± SD obtained by triplicate analyses.
[b] ND = not detected.
[c] Values bearing different letters in the same row are significantly different ($p < 0.05$).

Pęksa *et al.*[17] reported that greater influence of harvest time on bioactive compounds was due to environmental and weather conditions such as low air temperature.

From the data[23] of Cetral Weather Bureau, Taiwan, we could obtain that the monthly mean temperatures in Keelung City in Nov. 2006, Dec. 2006, Jan. 2007, Feb. 2007 and Mar. 2007 were 22.1, 18.4, 16.5, 17.8 and 19.0°C, respectively. By compared with our results, the saponin contents in yam seem to increase with the reduced temperature (from Nov. 2006 to Jan. 2007) and then decrease with raised temperature (from Jan. 2007 to Mar. 2007). Yam gathered at the lowest temperature (Jan. 2007) had the highest saponin content. Dong *et al.*[21] found that harvest time would influence saponin contents in roots of *Panax notoginseng* cultivated in China. Pecetti *et al.*[22] demonstrated that climatic temperature was the main factor affecting saponin contents in lucerne (*Medicago sativa* L.). Their results were similar to ours.

Journal of Food and Drug Analysis, Vol. 17, No. 2, 2009

Table 5. Saponin contents in yam vines

Compounds	Saponin contents (μg/g dw)[a]				
	Harvest time				
	Nov. 2006	Dec. 2006	Jan. 2007	Feb. 2007	Mar. 2007
1	ND[b]	ND	ND	ND	ND
2	6.82 ± 1.25 C[c]	8.24 ± 1.22 A	7.98 ± 1.15 AB	7.30 ± 1.82 BC	4.36 ± 0.79 D
3	ND	5.09 ± 0.88 A	5.19 ± 1.31 A	ND	ND
Total furostanol glycosides	6.82 ± 1.25 B	13.33 ± 2.10 A	13.17 ± 2.46 A	7.30 ± 1.82 B	4.36 ± 0.79 C
4	ND	ND	ND	ND	ND
5	4.70 ± 0.89 C	7.07 ± 1.34 A	7.42 ± 1.08 A	5.92 ± 0.94 B	3.78 ± 0.52 D
6	ND	4.55 ± 0.48 A	4.47 ± 0.65 A	ND	ND
Total spirostanol glycosides	4.70 ± 0.89 C	11.62 ± 1.82 A	11.89 ± 1.73 A	5.92 ± 0.94 B	3.78 ± 0.52 C
Total saponins	11.52 ± 2.14 B	24.95 ± 3.92 A	25.06 ± 4.19 A	13.22 ± 2.76 B	8.14 ± 1.31 C

[a]. All values are mean ± SD obtained by triplicate analyses.
[b]. ND = not detected.
[c]. Values bearing different letters in the same row are significantly different ($p < 0.05$).

CONCLUSIONS

Harvest time would influence saponin content in yam. Environmental temperature might play a crucial factor. Yam harvested in January with the lowest monthly mean temperature had higher saponin content than those gathered in other months. Regardless of harvest time, tuber cortex and flesh, the reproductive organs of yam contained much higher saponin than rhizophor, leaf and vine; furthermore, tuber cortex had the highest saponin level.

ACKNOWLEDGMENTS

This research was supported by the National Science Council, Taiwan (project no. NSC 95-2313-B-040-002) and the Chung Shan Medical University, Taiwan (project no. CSMU-95-OM-A-093).

REFERENCES

1. Hu, K., Dong, A., Yao, X. S., Kobayashi, H. and Iwasaki, S. 1996. Antineoplastic agents. I. Three spirostanol glycosides from rhizomes of *Dioscorea collettii* var. hypoglauca. Planta Med. 62: 573-575.
2. Hu, K., Yao, X., Kobayashi, H. and Iwasaki, S. 1997. Antineoplastic agents. II. Four furostanol glycosides from rhizomes of *Dioscorea collettii* var. hypoglauca. Planta Med. 63: 161-165.
3. Yang, D. J., Lu, T. J. and Hwang, L. S. 2003. Isolation and identification of steroidal saponins on Taiwanese yam cultivar (*Dioscorea pseudojaponica* Yamamoto). J. Agric. Food Chem. 51: 438-444.
4. Hu, C. C., Lin, J. T., Liu, S. C. and Yang, D. J. 2007. A spirostanol glycoside from yam (*Dioscorea villosa*) extract and its cytistatic activity on three cancer cells. J. Food Drug Anal. 15: 310-315.
5. Peng, J. P., Chen, H., Qiao, Y. Q., Ma, L. R., Narui, T., Suzuki, H., Okuyama, T. and Kobayashi, H. 1996. Two new steroidal saponins from *Allium sativum* and their inhibitory effects on blood coagulability. Acta Pharm. Sinica 31: 613-616.
6. Aquino, R., Conti, C., DeSimone, F., Orsi, N., Pizza, C. and Stein, M. L. 1991. Antiviral activity of constituents of *Tamus communis*. J. Chemother. 3: 305-309.
7. Santos, W. N., Bernardo, R. R., Pecanha, L. M. T., Palbtnik, M., Parente, J. P. and deSousa, C. B. P. 1997. Haemolytic activities of plant saponins and adjuvants. Effect of *Periandra mediterranea* saponin on the humoral response to the FML antigen of *Leishmania donovani*. Vaccine 15: 1024-1029.
8. Sauvaire, Y., Ribes, G., Baccou, J. C. and Loubatieŕes-Mariani, M. M. 1991. Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek. Lipids 26: 191-197.
9. Kato, A., Miura, T. and Fukunaga, T. 1995. Effects of steroidal glycosides on blood glucose in normal and diabetic mice. Biol. Pharm. Bul. 18: 167-168.
10. Šavikin-Fodulovic, K., Grubisic, D., Culafic, L., Menkovic, N. and Ristic, M. 1998. Diosgenin and phytosterols content in five callus lines of *Dioscorea balcanica*. Plant Sci. 135: 63-67.
11. Chen, Y., and Wu, Y. 1994. Progress in research and manufacturing of steroidal sapogenins in China. J.

Herb. Spic. Med. Plants 2: 59-70.
12. Dinan, L., Harmatha, J. and Lafont, R. 2001. Chromatographic procedures for the isolation of plant steroids. J. Chromatogr. A 935: 105-123.
13. Lin, J. T. and Yang, D. J. 2008. Determination of steroidal saponins in different organs of yam (*Dioscorea pseudojaponica* Yamamoto). Food Chem. 108: 1608-1074.
14. Liu, S. Y., Wang, J. Y., Shyu, Y. T. and Song, L. M. 1995. Studies on yams (*Dioscorea* spp.) in Taiwan. J. Chin. Med. 6: 111-126.
15. Yang, D. J., Lu, T. J. and Hwang, L. S. 2003. Simultaneous determination of furostanol and spirostanol glycosides in Taiwanese yam (*Dioscorea* spp.) cultivars by high-performance liquid chromatography. J. Food Drug Anal. 11: 10-15.
16. Mambelii, S, and Grandi, S, 1995. Yield and quality of kenaf (*Hibicus cannabinus* L.) stem as affected by harvest date and irrigation. Ind. Crop Prod. 4: 97-104.
17. Pęksa, A., Golubowska, G., Rytel, E., Lisińska, G. and Aniolowski, K. 2002. Influence of harvest date on glycoalkaloid content of three potato varieties. Food Chem. 78: 313-317.
18. Kraverts, S. D., Vollerner, Y. S., Gorovits, M. B. and Abubakirov, N. K. 1990. Steroids of the spirostan and furostan series from plants of the genus *Allium*. Chem. Nat. Comp. 26: 359-373.
19. Milgate, J. and Roberts, D. C. K. 1995. The nutritional and biological significance of saponins. Nutr. Res. 15: 1223-1249.
20. Amaducci, S. and Pritoni, P. 1998. Effect of harvest date and cultivar on *Cichorium intybus* yield components in north Italy. Ind. Crop Prod. 7: 345-349.
21. Dong, T. T. X., Cui, X. M., Song, Z. H., Zhao, K. J., Ji, Z. N., Lo, C. K. and Tsim, K. W. K. 2003. Chemical assessment of roots of *Panax Notoginseng* in China: regional and seasonal variations in its active constituents. J. Agric. Food Chem. 51: 4617-4623.
22. Pecetti, L., Romani, T. M., Denedetto, M. G. and Corsi, P. 2006. Variety and environment effects on the dynamics of saponins in lucerne (*Medicago sativa* L.) Europ. J. Agronomy 25: 187-192.
23. Central Weather Bureau, Taiwan. Data for Monthly Mean Temperature. <http://www.cwb.gov.tw/ >